**Supporting information**

# Enhancing phytate availability in soils and phytate-P acquisition by plants: a review

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**Table S1** Table 1 continued. (A) $P_o$ fractions (labile, microbial, chemisorbed, internal, occluded; mg kg$^{-1}$) in cultivated (+P) and uncultivated (-P) two volcanic soils (Vilcun and Osorno) and two forest watershed soils (broadleaf and coniferous) determined by sequential extraction. (B) EDTA-extractable phytase-hydrolyzable P (EDTA-$P_{Phy}$) in different soil aggregates and animal wastes.

**(A)**

| **Soil series** | **Soil description** | **Labile $P_o$ ($NaHCO_3$)** | **Microbial $P_o$ ($NaHCO_3/CHCl_3$)** | **Chemisorbed $P_o$ (NaOH)** | **Internal $P_o$ (Ultrasonic NaOH)** | **Ocluded $P_o$ (HCl)** | **$P_t$** | **Acid $P_o$ (%$P_t$)** | **Reference** |
|---|---|---|---|---|---|---|---|---|---|
| Vilcun, Chile | cultivated (+P) | 19 | 25 | 1436 | 703 | 217 | – | – | Hedley et al.[1] |
| | uncultivated (-P) | 13 | 21 | 943 | 673 | 172 | – | – | |
| Osorno, Chile | cultivated (+P) | 85 | 59 | 2161 | 588 | 41 | – | – | |
| | uncultivated (-P) | 34 | 32 | 110 | 548 | 26 | – | – | |
| Chiloé, Chile | broadleaf forest watershed | 69.2±6.2 | – | 121±23.1 | – | – | 413±33 | 34.8±5.3 (54) | Thomas et al.[2] |
| | coniferous forest watershed | 46.3±4.4 | – | 89.7±7.4 | – | – | 357±25 | 56.5±8.0 (54) | |

**(B)**

| **Soil series** | **Soil description** | **EDTA-extractable phytase hydrolyzable P (EDTA-$P_{Phy}$; mg kg$^{-1}$)** | **Aggregate size (mm) and % of bulk soil P-fraction concentration** | | | | | **Reference** |
|---|---|---|---|---|---|---|---|---|
| | | Bulk soil | >2.00 | 0.50–2.00 | 0.21–0.50 | 0.053–0.21 | <0.053 | |
| no-till (NT) | lower nutrient erosion | 96.1 | 101 | 109 | 104 | 98 | 73 | Green[3] |
| chisel-till | mineral fertilized | 81.1 | 92 | 105 | 101 | 86 | 78 | |
| organic (ORG) | moldboard plow system receiving animal manures | 72.6 | 101 | 113 | 108 | 86 | 84 | |

| **Animal wastes** | **$P_{phy}$** | **%$P_t$** | **%$P_o$** | **Experimental conditions: Extraction reagents** | | **Experimental conditions: Enzyme** | **Reference** |
|---|---|---|---|---|---|---|---|
| Cattle manure | 169 | 9.4 | | Sequential $H_2O$, $NaHCO_3$, NaOH | | a–d** | He and Honeycutt[4] |
| | 698 | | | $H_2O$ | | a | Dao[5] |
| | 281 | 5.2 | 25 | Sequential $H_2O$, $NaHCO_3$, NaOH | i | a–d | He et al.[6] |
| | 140 | 2.6 | 8.7 | | ii | | |
| | 185 | 23.5 | 60 | Sequential $H_2O$, $NaHCO_3$, NaOH | | b, e | He et al.[7] |
| | 3811 | 32.2 | | EDTA | | a | Dao et al.[8] |
| | 1286 | 18.8 | 75 | NaOH-EDTA | iv | a–d | He et al.[9] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 472 | 6.7 | 95 | | v | | |
| | 417 | 9.7 | | $H_2O$ | | a, e | |
| | 1311 | 19.7 | | 100 mM NaOAc pH 5.0 | | | |
| | 708 | 10.9 | | 100 mM NaOAc, 50 mM EDTA | | | |
| | 1436 | 27.3 | | 1 M HCl | | | He et al.[10] |
| | 1245 | 18.6 | | 0.25 M NaOH, 50 mM EDTA | | | |
| | 1629 | 25.8 | | 0.5 M NaOH, 50 mM EDTA | | | |
| Mean | 1047 | 16.7 | 53 | | | | |
| | 705 | 33.6 | 38 | | | a–d | He and Honeycutt[4] |
| Swine manure | 486 | 10.1 | 76 | Sequential $H_2O$, $NaHCO_3$, NaOH | iii | a–d | He et al.[6] |
| | 277 | 5.8 | 20 | | | | |
| | 360 | 13.2 | 23 | | | b, e | He et al.[11] |
| Mean | 457 | 15.7 | 39 | | | | |
| | 2198 | 16.3 | 84 | NaOH-EDTA | iv | a–d | He et al.[9] |
| | 2171 | 17.5 | 83 | | v | | |
| | 218 | 4.5 | | $H_2O$ | | a, e | He et al.[10] |
| Poultry manure | 573 | 7.5 | | 100 mM NaOAc pH 5.0 | | | |
| | 2153 | 16.2 | | 100 mM NaOAc, 50 mM EDTA | | | |
| | 4209 | 29.0 | | 1 M HCl | | | |
| | 2970 | 22.1 | | 0.25 M NaOH, 50 mM EDTA | | | |
| | 3727 | 26.0 | | 0.5 M NaOH, 50 mM EDTA | | | |
| Mean | 2277 | 17.4 | 84 | | | | |
| | 170 | 5.3 | | $H_2O$ | | a, e | He et al.[10] |
| | 8258 | 63.3 | | 100 mM NaOAc pH 5.0 | | | |
| Poultry litter | 10140 | 65.5 | | 100 mM NaOAc, 50 mM EDTA | | | |
| | 9710 | 64.2 | | 1 M HCl | | | |
| | 11085 | 70.7 | | 0.25 M NaOH, 50 mM EDTA | | | |
| | 11091 | 70.7 | | 0.5 M NaOH, 50 mM EDTA | | | |
| Mean | 8409 | 56.6 | | | | | |

** (a) *Aspergillus ficuum* phytase, (b) wheat phytase, (c) wheat germ acid phosphatase, (d) Bovine intestinal mucosa alkaline phosphatase and (e) Potato acid phosphatase, (i) Fresh, (ii) after one year storage 22˚C, (iii) after one year storage 4˚C (iv) wet and (v) dry.[12]

**Table S2** Table 3A continued. Summary of known plant secreted organic acids to mobilize soil P.

| Plant family/species | Location and soil P (mg kg$^{-1}$) | | | | Total carboxylates (μmol g$^{-1}$ root dw) | Root organic acid species and %Total carboxylates | | Reference |
|---|---|---|---|---|---|---|---|---|
| | Location | $P_t$ | bicarb.-extr. P | P retention index* | | citric | malic | |
| field pea (*Pisum sativum*) | Bindoon | 133 | 5 | 33.8 | 1.5–5 | 74–98 | | Nuruzzaman et al.[12] |
| | Mingenew | 119 | 9 | 58.1 | 12.5–15 | 98 | – | |
| | Nyabing | 70 | 6.5 | 7.2 | 25–50 | 97–99 | | |
| white lupin (*Lupinus albus*) | Bindoon | | | | 15–18.8 | 62.5–87.5 | 18.8–43.8 | |
| | Mingenew | | | | 18.8–25 | 50 | 56.3 | |
| | Nyabing | | | | 37.5–50 | 68.8–74.4 | 37.5 | |
| faba bean (*Vicia faba*) | Bindoon | | | | 2–5 | | | |
| | Mingenew | – | – | – | 2.75–9.75 | – | – | |
| | Nyabing | | | | 6.25–14.5 | | | |
| wheat (*Triticum aestivum*) | Bindoon | | | | 0–2.25 | | | |
| | Mingenew | | | | 5–12.5 | – | – | |
| | Nyabing | | | | 7.5–10 | | | |

| Plant family/species | Root organic acid species | Organic acid concentration | | P-mobilizing capacity | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Soil initial P | Soil mobilized P (μmol g$^{-1}$) | | | | |
| **Fabaceae** | | mmol g$^{-1}$ root | μmol g$^{-1}$ soil | | $H_2O$-extr.[b] | CAL[e] | Olsen | Soil solution (mg L$^{-1}$) | |
| *Lupinus albus* L. | citric | 0.24 fw | 47.7 | – | | >5.5 | | | Dinkelaker et al.[13] |
| | | 8.8–22.1 fw | 47.7±7.2 | | 61±7 | 581±76 | 484±68 | 12.5 | Neumann et al.[14] |
| *Lupinus angustifolius* | | 60 dw | | | | | | | White and Robson[15] |
| *Lupinus consentinii* | | 85 dw | – | | | – | | | |
| pea (*Pisum sativum*) | | 90 dw | | | | | | | |
| pigeon pea (*Cajanus cajan* L. Millsp.) | piscidic | | | Fe-bound P | | | | | Dao[16] |

| Plant family/species | Root organic acid species | Organic acid efflux | | Reference |
|---|---|---|---|---|
| | | nmol g[−1] fw h[−1] | units shown | |
| *Brassica napus* | malic | 200 | 0.43 nmol cm[−1] root h[−1] | Hoffland et al. [17] |
| | citric | 70 | 0.14 | |
| rice | citric | 337 | | Kirk et al.[18] |
| *Lupinus albus* | citric | 570, 1160, 2380 | | Johnson et al.,[19] Neumann et al.,[14] Keerthisinghe et al.[20] |
| | malic | 510, 130 | | |
| *Alfalfa* | citric | 3.5 | | Lipton et al.[21] |
| maize | malic | 430 | | Jones and Darrah[22] |
| | citric | 90 | | |
| wheat | malic | 4000 | 2 nmol apex[−1] h[−1] | Ryan et al.[23] |
| maize | citric | 55 | 0.25 | Pellet et al.[24] |
| tobacco | citric | 240 | 0.18 | Delhaize et al.[25] |
| chickpea | malonic | | 2 nmol plant[−1] h[−1] | Ohwaki and Sugahara[26] |
| | tartaric | | 0.4 | |
| | citric | | 0.4 | |
| | fumaric | | 0.4 | |
| | | mmol g[−1] fw s[−1] | | |
| Harsh hakea (*Hakea prostrata* R.Br.) | malic | 0.05–0.34 | | Shane et al. [27] |
| | citric | 0.01–0.04 | | |
| | cis-aconitic | 0–0.04 | | |
| | trans-aconitic | 0–0.17 | | |
| | lactic | 0–0.13 | | |

| Plant species | P-mobilizing capacity (mg kg[−1]) | | | | Reference |
|---|---|---|---|---|---|
| | Al-P | Fe-P | Ca-P | labile-P | |
| Buckwheat (*Fagopyrum esculentum*) | | | 121-126 | | Teboh and Franzen[28] |
| spring wheat (*Triticum aestivum*) | | | 127-135 | | |
| ruzigrass (*Urochloa ruziziensis*) | 0.00–0.05 | 363-484 | anion exchange resin (AER) | | Almeida and Rosolem[29] |
| | 0.05–0.10 | 385-465 | $NaHCO_3$-extr. | | |

[*] Phosphorus retention index, was estimated as the ratio between sorbed P and solution P after equilibration of 2.5 g soil with 50 mL of 0.02 M KCl containing 10 mg $L^{-1}$ P.[13]

[b] bicarb.-extr. = bicarbonate-extractable; extr. = extractable. Bicarbonate-extractable P is extracted with 0.5 M sodium bicarbonate at pH 8.5.[13]

[e] CAL–0.13 mmol calcium acetate lactate extractable P $kg^{-1}$ soil.[31]

**Table S3** Transgenic plant or yeast, phytase genes source and the expressed phytase activity and properties.

| Transgenic plant | Phytase gene source | Gene sequence | Specific activity | | pH optim. | Temp. optim. (˚C) | $K_m$ (µM) | Catalytic efficiency $K_{cat}$ ($s^{-1}$) | Thermal stability | $M_w$ (kDa) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | U $mg^{-1}$ | µKat $mg^{-1}$ | | | | | | | |
| Potato (*Solonum tuberosum*) | *Aspergillus ficuum* (*niger*) | | 180 | 3 | 5.0 | 58 | 124 | | | 67.5–81.6 | Ullah et al.[30] |
| Soybean (*Glycine max*) | *Aspergillus niger* | initiation codon: 5'-GCGTCTAGATGCTGGCAGTCCCCGCCTC-3<br>Upstream oligonucleotide: 5'-GCGTCTAGACTGGCAGTCCCCGCCTCG-3'<br>downstream oligonucleotide: 5'-TGCTCTAGACTAAGCAAAACACTCCG-3 | 55.2 | 0.92 | 58˚C (pH 3.0, 5.5), 63˚C (pH 5.0), 66˚C (pH 4.5), highest activity at 63˚C and pH 5.0 | | | | | 69–71 | Li et al.[31] |
| Tobacco (*Nicotiana tabacum* L. cv.) NC89 | *Agrobacterium tumefaciens* LBA4404 | The gene sequence *phy*AI was shown on the record as GenBank Accession: AY013315. | | | 2.0, 5.5 | 50 | 730 (Na-phytate), 1300 (Ca-phytate) | $1.2\times10^4$ (Na-phytate), $5.1\times10^3$ (Ca-phytate) | 25.1% residual activity at 80˚C for 15 min | 76 | Zhang et al.[32] |
| Alfalfa (*Medicago sativa*) | *Aspergillus ficuum* | | 226 | 3.76 | 5.0 | 58 | 50 | | Inactivated completely at 68˚C | 73–100 | Ullah et al.[33] |

| Transgenic plant or yeast | Phytase gene source | Gene sequence | Specific activity | pH optim. | Temp. optim. (˚C) | Thermal stability | $M_w$ (kDa) | Reference |
|---|---|---|---|---|---|---|---|---|
| Rice (*Oryza sativa*) | Yeast (*Schwanniomyces occidentalis*) | full-length codon-modified phytase | 4.6 U[a] $g^{-1}$ fw[b] | pH 4.5 determined at 37˚C | 70˚C determined at pH 5.5 | lost 32% and 92% activity at 80˚C and 90˚C | 70 | Hamada et al.[34] |
| | | truncated codon-modified phytase | 10.6 U $g^{-1}$ fw | pH 5.0 determined at 37˚C | 50–60˚C determined at pH 5.5 | lost 87% and 94% activity at 70˚C and 80˚C | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Yeast (*Aspergillus oryzae*) A1560 | *Peniophora lycii* | http://www.expasy.ch/cgi-bin/get-prodoc-entry?PDOC00538 grouped as 6-phytases (EC 3.1.3.26) | 1080±110 U mg$^{-1}$ protein | 4.0–4.5 | 50–55 | 62%[c] | 72 | Lassen et al.[35] |
| | *Agrocybe pediades* | | 400 | 5.0–6.0 | 50 | 47% | 59 | |
| | *Ceriporia* sp. 1 | | 700±80 | 5.5–6.0 | 55–60 | 38% | 59 | |
| | *Ceriporia* sp. 2 | | 1040±310 | 5.0–6.0 | 40–45 | 22% | 54 | |
| | *Trametes pubescens* | | 1210±30 | 5.0–5.5 | 50 | 15% | 62 | |
| | *Aspergillus niger* | | 100 | 2.5–5.5 | 50 | 52% | – | |
| Saccharomyces cerevisiae | *Aspergillus niger* | – | 4.0 U mg$^{-1}$ | 2–2.5, 5–5.5 | 55–60 | – | 120 | Han et al.[36] |
| Methylotrophic yeast (*Pichia pastoris*) | *Aspergillus niger* | – | 25–65 U mL$^{-1}$ | 2.5, 5.5 | 60 | – | 95 | |

| **Transgenic plant** | **Phytase gene source** | **Gene sequence** | **Transgenic lines** | **Phytase expression (FTU g$^{-1}$)** | | **M$_w$ (kDa)** | **Reference** |
|---|---|---|---|---|---|---|---|
| | | | | **Average** | **Highest** | | |
| Canola (*Brassica napus*) | *Aspergillus niger* | two oligos: 5'AGGATCCATGGACTTGAAATCTTTCCCATTC3'; 5'ACGAGCTCTTAAGCAAAGCATTCAGCCCAATCA CCAC3' | Native phytase without KDEL[d] (n[e]=8) | 7.6 | 15 | 70 | Peng et al.[37] |
| | | | Codon-modified phytase without KDEL (K0) (n=32) | 8.8 | 21 | | |
| | | the upper strand primer: 5'AGGATCCATGGACTTGAAATCTTTCCCATTCTATGCTTTCTTGTGTTTCGGTCAATATTTCGTTGCTGTTACTCATGGACTGGCAGTCCCCGCCTCGAG3' | Native phytase with KDEL (n=26) | 10.3 | 24 | | |
| | | | Codon-modified phytase with KDEL (MPHY2) (n=103) | 15.6 | 41 | | |

[a] One unit (U, μmol mg$^{-1}$) of phytase activity was defined as the amount of phytase required to hydrolyze sodium phytate to produce 1 μmol P per min at 37°C and pH 5.5.[34,36]

[b] fw = fresh weight.

[c] Residual activities were measured after preincubation of the enzymes for 60 min at 80°C in 0.1 M sodium acetate, pH 5.5 (Lassen et al., 2001).

[d] KDEL=Lys-Asp-Glu-Leu.

[e] n = number of transgenic tested.

[f] KDEL=Lys-Asp-Glu-Leu.

[g] n = number of transgenic tested.

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